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Biomaterials

Development of membranes for the cultivation of kidney epithelial cells

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Abstract

The development of biohybrid organs (BHO) will benefit from improved membranes regarding transport and cell contacting properties. Here we describe in a first study the development and testing of membranes made of polyacrylonitrile (PAN) and polysulfone (PSU) for the immobilisation of kidney epithelial cells. Comparative investigations on overall polymer toxicity tested with 3T3 fibroblasts, and morphology and proliferation of Madin-Darby canine kidney (MDCK) cells cultured on the membranes could show that these materials have comparable cell contacting properties like Millicell ™ membranes. Since PAN and PSU have superior membrane forming properties with regard to membrane geometry, i.e. for the preparation of hollow fibres, and porosity, i.e. for immuno isolation, both materials or modifications thereof seem to be suitable for the application in BHO such as biohybrid kidney. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: Biohybrid organs; Polymer membranes; Kidney epithelial cells; Biocompatibility

1. Introduction

Kidney was the first organ whose function was substituted by an artificial device [1], and the first organ to be successfully transplanted [2,3]. Kidney's functions are to eliminate the water-soluble nitrogenous end-products of protein metabolism, to maintain electrolyte balance in body fluids and to excrete the excess electrolytes, to contribute to obligatory water loss and discharge excess water in urine and to maintain the acid–base balance in body fluids and tissues. Each kidney is made up of over a million parallel mass transfer units called nephrons, and can be viewed as a sequential arrangement of mass transfer devices in which the glomerulus acts as filtering unit, and the proximal and distal tubules as the regulatory or reabsorptive units, for two fluid streams, namely blood and urine [4].

In the event of acute or chronic renal failure, the major danger is the impurification of the blood from accumulated compounds, such as toxins, salts, and water. This problem has been partly resolved by hemodialysis that replaces the ultrafiltration function of the glomerulus through diffusive and convective transport of these compounds across a membrane. A high number of patients with end-stage renal failure (ESRD) have been successfully treated with hemodialysis (HD) for many years now [4]. However, long-term HD treatment is associated with an increase in morbidity and mortality in patients [5,6]. Patients with ESRD under long-term dialysis are known to suffer from immuno deficiency that leads to increased susceptibility to infections, a higher incidence of malignant tumours, a decrease in cutaneous delayed hypersensitivity, and defective responses to T cell-dependent antigens [7,8]. Long-term HD also promotes the onset of the carpal tunnel syndrome, caused by β_2-microglobulin (β_2-m) amyloid deposits in the synovium through the activation of monocytes and macrophages leading to the release of cytokines and proteases [9]. About 50% of ESRD patients develop dialysis-related amyloidosis after 10 yr of treatment [10]. Normally, β_2-m is filtered through the glomerular basement membrane of the kidney, reabsorbed and degraded by the renal tubular cells. However, conventional dialysis membranes, and even porous high-flux membranes, are poorly permeable to β_2-m [11–13].

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This explains the necessity to develop a biohybrid kidney that replaces the critical elements of renal function, including the excretory, reabsorptive, metabolic and endocrine functions [14]. A first possible model for a biohybrid kidney was described combining the ultra filtration ability of conventional dialysis membranes with a subsequent back filtration by functioning kidney epithelial cells [15–17]. The active transport mechanism from the ultrafiltrate of the glomerulus is performed by the renal tubular cells; this establishes electrochemical gradients for specific solutes [15,16]. Two cell lines were proposed that could be used in a biohybrid kidney, namely LLC-PK1 cells as proximal renal tubule cells of the Hampshire pig kidney [17], and MDCK [Madin–Darby canine kidney] cells as distal or cortical collecting tubule cells [18]. Prerequisites for the construction of such a device are that renal epithelial cells must be able to attach, proliferate and function in a polarised monolayer with apical microvilli on a permselective membrane. This cell layer must be impermeable to non-specific solute movements, i.e. tight junctions must be formed and transepithelial transport must be controlled by the cells [19–21]. For application in the body as a biohybrid organ, the membrane also has to protect allogenic or xenogenic cells from the immune system to avoid host-versus-graft reactions. Therefore, the membrane ought to have a cut-off of about 100 kDa to prevent the permeation of immunoglobulins and complement factors [22]. Polymers used for the preparation of membranes in conventional bioartificial kidneys such as polyacrylonitrile (PAN) and polysulfone (PSU) have the advantage of a good membrane formation of flat membranes and hollow fibres with controlled pore diameters. The possibility of sterilising PSU by steam and the ease of surface functionalisation of PAN with various chemical groups might be considered as advantages for a possible application of these polymers in biohybrid organs. However, there is only very limited knowledge about their biocompatibility versus tissue cells.

The development of a biohybrid kidney, composed of a separating membrane for passive removal of solutes and kidney epithelial cells for active functions, may benefit from the optimisation of membrane properties with respect to the transport functions and tissue compatibility. In the present investigation we have compared the growth and the morphology of MDCK cells on flat PAN and PSU membranes, to learn more about the dependence of kidney epithelial cell growth and function on the underlying membrane. Details are reported herein.

2. Materials and methods

2.1. Membranes

PAN and PSU (type: Udel 3500, Amoco Chemicals, Germany) asymmetric flat membranes prepared by phase inversion technique were investigated in this study. PSU membranes were blended with polyvinylpyrrolidone (PVP, type: K 25, Fluka, Germany) as described below. The commercial Millicell-HA cellulose mix-esters (MC) (Millipore, Germany) symmetric membrane was investigated as a control membrane.

The flat membranes were formed by a so-called bicomponent process [23], whereby two chemically different, incompatible polymer solutions were cast subsequently onto a glass support with a casting knife and coagulated in a common nonsolvent. A two-layer-shaped membrane composite was formed which can easily be separated into two polymer membranes. During the formation of the PAN membranes first the glass support was coated with a 12 wt% polymer solution of cellulose acetate in N,N-dimethylformamide (DMF; layer thickness: 0.3 mm), followed by coating with a 10 wt% polymer solution of PAN in DMF (layer thickness: 0.3 mm). The solution composite was coagulated in water for 30 min, separated into two membrane components, and the membrane of interest was washed with water free of solvent. PAN-membranes with a highly asymmetric morphology with a relatively dense surface at the polymer solution/water interface and a highly porous surface at the interface between both different polymer solutions were formed by this process.

PSU membranes with an asymmetric structure were prepared in a similar way but the glass support was coated with a 25 wt% polystyrene solution in N,N-dimethylacetamide (DMAc) followed by a 15 wt% PSU blend solution as the top layer. The PSU blend solution was composed of 15 wt% PSU, 15 wt% PVP K 25 and 70 wt% DMAc. Besides the high asymmetric structure of PSU membranes, the membrane wall morphology was similar to that of PAN membranes.

2.2. Characterisation with scanning electron microscopy (SEM)

The membrane samples were dehydrated in graded ethanols 50, 70, 80, 90, 96 wt%, twice with absolute ethanol and finally with ether. Such treated flat membranes were broken under liquid nitrogen, attached to sample supports and coated with a gold layer.

2.3. Characterisation of the pore diameter

Two different methods were used for the characterisation of pore diameters: permeation of solutes with specific molecular weight and subsequent analysis of the permeate by (i) gel permeation chromatography (GPC) and (ii) the bubble point method with or without pressure. The first technique involves the filtration of a solution of dextrans of specific molecular weights. The initial solution was passed through the membrane (feed) and was analysed as the filtrate solution (permeate) with GPC so

that the membrane pore diameter and cut-off could be calculated [24]. The bubble point technique is based on the liberation of a gas pressured through the dry membrane and another through the liquid-saturated membrane. The Young–Laplace theorem gives the relation between the pressure the gas needs to pass through the membrane and the diameter of the pores [25].

2.4. Spin coating of polymer films

Glass slides were cleaned with an ethanol/acetone mixture (1 : 1, vol/vol) and air dried. A drop of a 2.5 wt% solution of PAN dissolved in DMF was placed on the slide which was then rotated at 2000 rpm for 2 min using a CONVAC 1001 apparatus (Convac, Germany). MC surface coatings were produced from a 5 wt% solution of Millicell-HA membranes dissolved in DMF as well. The thickness of the films was about 20 μm. PSU films were prepared from a 5 wt% PSU solution dissolved in DMF and prepared by hand with a casting knife. Then the films were dried in a vacuum, to avoid precipitation of the solution. Thickness of casting films was about 50 μm.

2.5. Contact angle measurements

The contact angles of the membranes were estimated by the captive bubble method on two different membrane samples per material and three different points per sample. The advancing and receding contact angles of the spin coating films were measured by the sessile drop method on two different slides, and on three different points on each glass slide per material.

2.6. Cells

MDCK (Madin–Darby Kidney Cells) obtained from ATCC (American Tissue Culture Collection) were grown in MEM Earle with L-glutamine medium (MEM) containing 10 (w/v) fetal bovine serum (FBS), 1 (w/v) Hepes (1 M) and 1 (w/v) antibiotics, in an humidified incubator with 5 vol% CO_2. All the chemicals were obtained from Biochrom Berlin, Germany. For experiments with spin coating films or membranes, MDCK cells were plated at a concentration of 80 000 cells ml^{-1}.

2.7. Cytotoxicity assays

PSU and PAN membranes were incubated in the medium without phenol red for 1, 2, 3 and 7 days. Cyanoacrylate resin Instantbond (Martha Langnas, Berlin, Germany) was used as positive cytotoxic control extracted as described above, while the medium was used as negative control. According to the recommendations of ISO 10993-5 [26], the extracts were layered on subconfluent NIH-3T3 fibroblasts with addition of 10% FBS. After a 24 h incubation, XTT assay (Boehringer Mann-

heim, Germany), to measure the activity of mitochondrial dehydrogenases [27], and the neutral red assay [28], estimating the integrity of the cell membrane, were carried out.

2.8. Proliferation studies

Proliferation of MDCK cells was measured after 1, 2, 3, 6 and 9 days by MTT assay (Boehringer Mannheim, Germany), a colorimetrical method for the quantification of cell growth and viability with a water insoluble reaction product [29]. Cells were incubated in MEM with 10 wt% FBS and mixed with (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) (MTT) reagent in the ratio 10 : 1. After 4 h of incubation the supernatant was carefully removed, without it showing any visible detachment of stained cells. The Formazan crystals formed were dissolved using 2-propanol and the OD of 150 μl was measured at 540 nm. Since the MTT assay depends on the metabolic activity of cells represented by the mitochondrial succinate dehydrogenase, a second technique had to be applied for the measurement of cell proliferation. The LDH assay (Boehringer Mannheim, Germany), a colorimetric assay based on the measurement of lactate dehydrogenase (LDH) activity released from the cytosol of lysed cells was used [30]. Cells were cultivated using the times indicated above. The cells were then washed twice with PBS and incubated with 1 wt% Triton X-100 in MEM without phenol red at room temperature for 90 min under shaking. The resulting cell lysate was centrifuged at $2000 \times g$ for 5 min. 100 μl of each sample was placed in a 96 well plate and mixed with 100 μl LDH solution. After a 30 min incubation in the dark, 50 μl HCl solution (1 N) was added per well to stop the reaction. The OD of 50 μl was then measured at 492 nm in duplicate for each sample.

2.9. Statistical analysis

The statistical analysis was done through an analysis of variance (ANOVA) and the determination of the significant differences according to Scheffe's model. A significant difference is observed when $P < 0.05$. The results are presented directly in the graphs.

2.10. Morphological investigations with fluorescence microscopy

MDCK cells were plated on PAN, PSU and MC spin coating films in MEM-Earle with 10 wt% FBS and cultivated in a humidified incubator with 5 vol% CO_2. Cell morphology was visualised by the addition of fluorescein diacetate solution. After a 5 min incubation at 37°C the slides were washed twice with PBS and observed with an inverted fluorescence microscope—Axiovert 100 (Carl Zeiss, Germany).

2.11. Morphological investigations with transmission electron microscopy (TEM)

The membranes with MDCK cells were fixed for 15 min with 3 wt% glutaraldehyde in PBS at 4°C. This was followed by rinsing with cacodylate buffer pH 7.2 for 3 × 2 min and post-fixation with OsO_4 (25 wt% in PBS) for 15 min at 4°C. The samples were rinsed three times with PBS buffer for 2 min at 4°C. This was followed by dehydration in graded ethanol of 70, 80, 90, 96 wt%, three times in absolute ethanol, 5 min each, soaking with propylene oxide (three times, 2 min), embedding with propylene oxide/Epon resin mixture (1 : 1) overnight. Ultrathin sections were cut with an Ultracut E Microtom (Reichert-Jung, Germany). Post-staining was carried out with 4 wt% uranylacetate for 10 min at 60°C. The samples were rinsed and dried, stained with lead citrate (5 wt%) and washed again. The examinations were performed with a Philips EM 410 transmission electron microscope (Phillips, The Netherlands).

3. Results

3.1. Measurement of the pores diameters

The results are summarised in Table 1 and were obtained by the permeation method or bubble point technique. The water permeability, the pore diameters and the cut-off measured reveal that MC is the most permeable membrane followed by PAN and PSU. Both PSU and PAN have pore diameters preventing the permeation of larger molecules such as immunoglobulins or complement factors. In contrast, MC is a typical microfiltration membrane which could not be used to protect xeno- or allogenic cells from the host immune system.

3.2. Scanning electron microscopy (SEM)

The morphology of the PAN membranes characterised by SEM demonstrated an asymmetric structure of the membrane with a very thin active layer which is visible in the cross section as shown in Fig. 1a. The pores of the active layer with a size of about 100 nm (see above) were not resolved by SEM with the applied magnification (Fig. 1b). Moreover, the cross section of the membrane demonstrates that there are a number of the so-called finger pores which reach from the active layer (smooth side) to the other very porous layer (rough side). The pore diameter of the rough side calculated from the SEM is larger than 50 μm as shown in Fig. 1c. Note the broad distribution of diameters and orientational changes of large pores in the rough side of the membrane.

On the contrary, PSU membranes possess a thicker active layer supported by a sponge intermediate layer.

Table 1
Separation properties of the membranes

Membrane	Water permeability $(l\,m^{-2}\,h^{-1}\,kPa^{-1})$	Middle pores diameter D_{50} (nm)	Cut-off D_{100} (nm)
Polysulfone	2.70	6.55[a]	19.75[a]
Polyacrylonitrile	38.77	80[b]	250[b]
Millicell-HA	294.5	810[c]	950[c]

[a] Permeation and GPC.
[b] Bubble point with high pressure (<40 bar).
[c] Bubble point with low pressure (<15 bar).

The cross section (Fig. 2a) also shows that the membrane has an asymmetric structure and the so-called finger pores as well. However, in contrast to the PAN membranes, the PSU membranes possess a sponge intermediate layer between the active layer and the beginning of the finger pores shown in the cross section. The smooth side shown in Fig. 2b which has pores with a diameter of about 10 nm (see above) that are not resolved at the applied magnification. At the rough side (Fig. 2c) the finger pores are partially open and have pore diameters between 10 to 20 μm.

3.3. Contact angle measurements

Contact angles of films and membranes were measured, respectively, with a sessile drop and a captive bubble methods, and are summarised in Table 2. The advancing water contact angles of PAN and PSU membranes were almost the same, while the MC membranes had about 20° higher contact angles. Receding water contact angles could not be estimated correctly for the membranes, because of their porous structure, which led to an irregular structure of the droplet during the retraction of water. Therefore no stable receding contact angle values were obtained. Water contact angles of spin coating films prepared from the same polymers expressed low hysteresis and slightly decreased advancing water contact angles for all materials.

3.4. Cytotoxicity assays

Membranes prepared from PAN and PSU expressed a non-toxic action on NIH-3T3 cells independent of the extraction time as shown in Fig. 3a and b. The metabolic activity of these cells measured with the XTT assay was not decreased upon contact with the extracts from both materials which was clearly observed during incubation with extracts prepared from the cyanoacrylate resin (Fig. 3a). The neutral red assay shows no detectable damage of the cell membrane integrity in the case of PAN and PSU extracts in contrast to the cyanoacrylate resin extracts which was applied as a positive control (Fig. 3b).
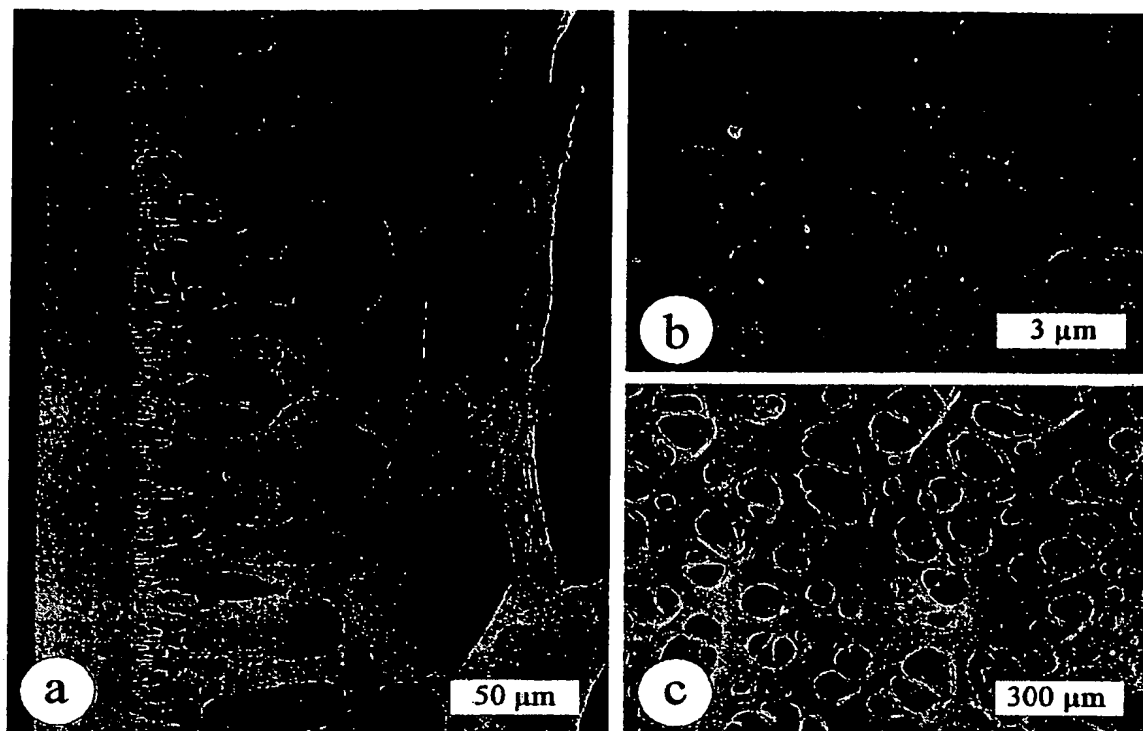
Fig. 1. Scanning electron microscopy of PAN membrane: (a) cross section; (b) smooth side and (c) rough side. White boxes are scale bars.
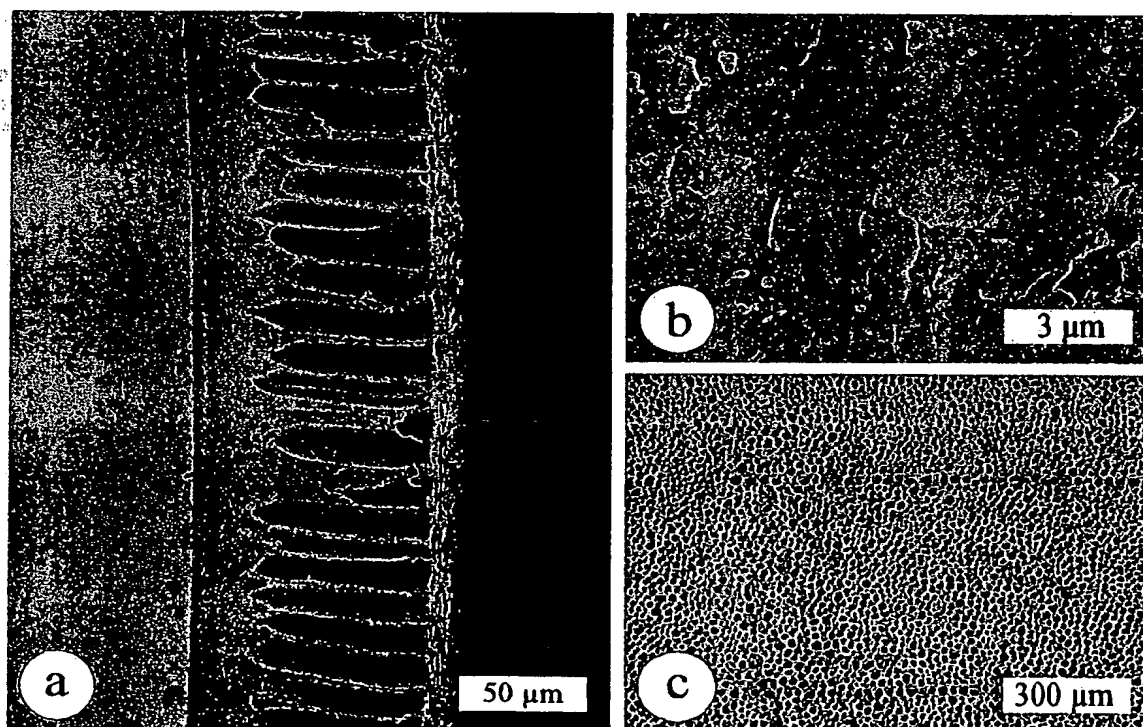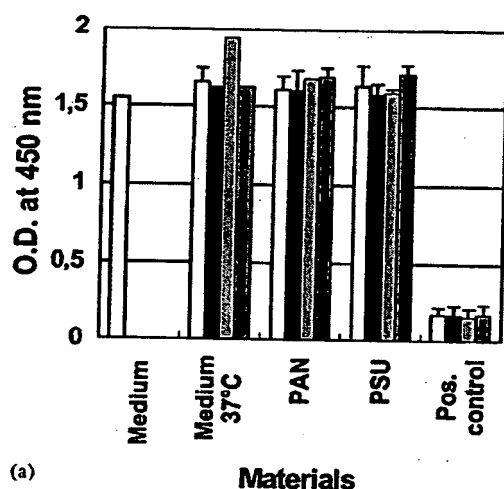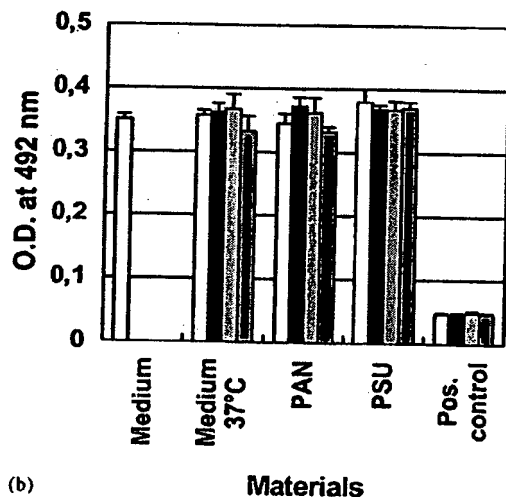

Fig. 2. Scanning electron microscopy of PSU membrane: (a) cross section; (b) smooth side and (c) rough side. White boxes are scale bars.

Table 2

Water contact angles for spin coating films and membranes

Material	Water contact angle (degree)	
	Advancing	Receding
Polyacrylonitrile (film)	60.4	38.0
Polyacrylonitrile (membrane)	62.7	—
Polysulfone (film)	52.4	37.0
Polysulfone (membrane)	39.6	—
Millicell-HA (film)	61.4	54.4
Millicell-HA (membrane)	83.4	—

(a)

(b)

Fig. 4. Metabolic activity of MDCK-C7 cells measured with the MTT assay as optical density (OD) function of the culture time on different membranes: (△) MC; (○) PAN; (□) PSU (mean ± SEM, $n = 12$ for PAN and PSU, $n = 6$ for MC): (a) MDCK cells grown on the smooth side of the membranes; (b) MDCK cells grown on the rough side of the membranes. Scheffe's statistical difference between: (◆) Millicell-HA and PAN; (●) Millicell-HA and PSU; (■) PAN and PSU; (◇) the three membranes. No significant difference: n.s.

(a) Materials

(b) Materials

Fig. 3. Cytotoxicity assays for 3T3 fibroblasts grown in contact with extracts of PAN, PSU and MC membranes after 1 (white bars), 2 (black bars), 3 (hatched bars) and 7 (dotted bars) days of extraction (mean ± SEM, $n = 6$). Cyanoacrylate is used as positive control, fresh and incubated medium as negative controls: (a) XTT-test; (b) Neutral Red-test.

3.5. Proliferation of MDCK cells

Cell proliferation was estimated by MTT assay after 1, 2, 3, 6 and 9 days of cultivation on MC, PAN and PSU membranes. Because of the asymmetric structure of PAN and PSU membranes, both smooth and rough sides were studied. The results in Fig. 4 show the OD averaged for about 12 samples per point at 562 nm, that is a measurement of the metabolic activity of the cells. Overall, it was demonstrated that the highest metabolic activity was observed for MDCK cells cultured on MC. Indeed, metabolic activity of cells on the smooth side of PAN and PSU was almost the same as for MC up to 6 days culture, but MC expressed the highest activity followed by PSU and PAN (Fig. 4a). In contrast, cells cultivated on the rough side of PSU demonstrated a significantly decreased metabolic activity followed by PAN in comparison to MC as shown in Fig. 4b. The LDH assay was applied to monitor the growth of cells on the different substrata shown in Fig. 5. Proliferation was studied on
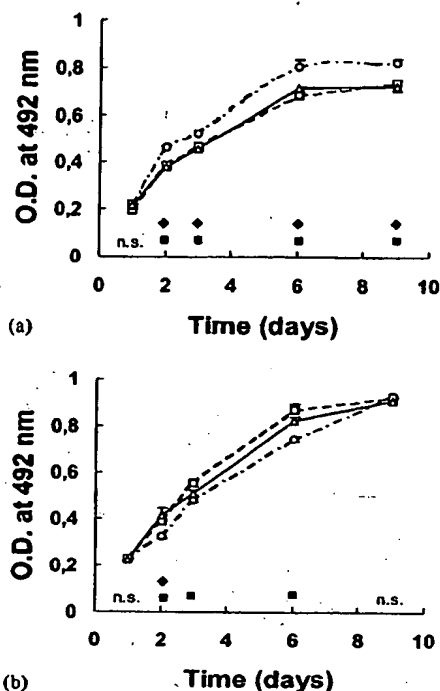
(a)

(b)

Fig. 5. Proliferation of MDCK-C7 cells cultured for 9 days on different support materials measured with LDH assay as optical density: (△) MC; (O) PAN; (□) PSU (mean ± SEM, n = 12 for PAN and PSU, n = 6 for MC). (a) MDCK cells grown on the smooth side of the membranes; (b) MDCK cells grown on the rough side of the membranes. Scheffe's statistical difference between: (♦) Millicell-HA and PAN; (■) PAN and PSU. No significant difference: n.s.

the smooth side (Fig. 5a) and the rough side (Fig. 5b) of the membranes, but yielded not only few differences between the different materials, but also insignificant differences between the smooth and rough sides. However, it was observed that the growth of cells had reached the plateau phase after six days of culture on the smooth side of the membranes, which was not evident during culture on the rough side.

3.6. Morphological investigations with fluorescence microscopy

The morphology of MDCK cells was studied after one day of culture on films with fluorescence microscopy, using fluorescein diacetate for labelling living cells. Fig. 6a shows that MDCK cells adhered and spread well on films made of MC membranes in a manner typical for this type of cell, with formation of larger cell aggregates with tight cell/cell contacts. A similar result was obtained for PAN as shown in Fig. 6b although the number and size of cell clusters seemed to be slightly lower. Also cells plated on PSU films did attach and spread (Fig. 6c).
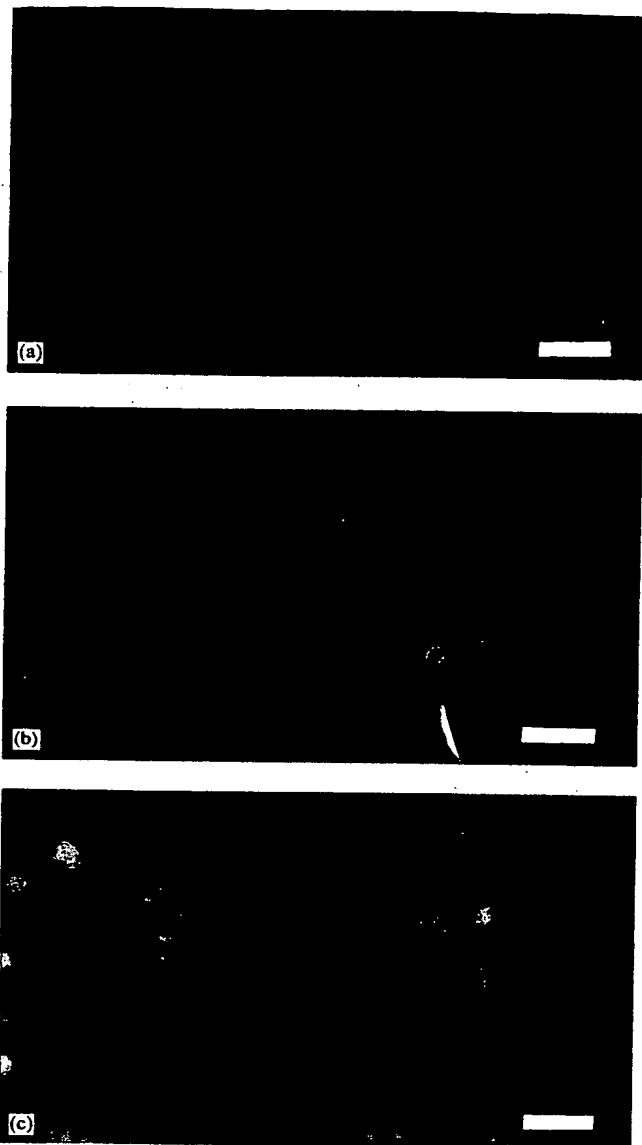

Fig. 6. Fluorosceine diacetate staining of MDCK cells cultured 24 h on different materials presented as spin coating films: (a) MC; (b) PAN; (c) PSU. Living cells present a green stained cytoplasma. Bar 20.8 μm.

3.7. Morphological investigations with transmission electron microscopy

Both sides of PAN and PSU membranes were examined by TEM. However, the cells cultured on the rough side of the membrane could not be documented because of the relation between the small field size in TEM and the large diameter of the pores in which the cells grew. Fig. 7a shows that MDCK cells growing on the smooth side of PAN were able to form very tight cell–surface contacts. Distances between the cell membrane and the polymer membrane surface were found to be less than

Fig. 7. Transmission electron micrograph of MDCK cells after 7 days of culture on the PAN membranes: (a) visualisation of strong attachment of epithelial cell sheet built up by MDCK cells on PAN support (S). Bar 1.3 µm; (b) presence of tight junctions (TJ) and microvilli (M) demonstrating orientation of cells, however weak cell–cell contacts along the basal region were observed. Bar 0.9 µm.

0.083 µm. While the cells approached the surface very closely, cell–cell contacts seemed to be less intensive. This was concluded from the fact that cells contacted each other only partly in the areas of tight junctions, but were separated from each other in other large areas (Fig. 7b). MDCK cells on PAN were also able to form microvilli as a second functional feature of epithelial cells (Fig. 7b). MDCK cells grown on the smooth side of PSU expressed microvilli in higher quantities as shown in Fig. 8a. However, it seems that the cell surface contact was not as stable as observed for PAN because the cell layer was separated from the underlying membrane by a distance of 1.16 µm. On the other hand cell–cell contacts were obviously more intensive than for PAN because it was difficult to visualise the border between adjacent cells which was only indicated by the high number of junctions (Fig. 8b).

4. Discussion

PAN and PSU are one of the most used synthetic materials for the production of dialyzer membranes.

Although their good blood contacting properties are well documented [31], much less is known about the compatibility of these materials with other tissues. Since membrane formation of these polymers is well established and porosity can be balanced more easily than with other materials, membranes made of PAN or PSU might be used for the development of biohybrid organs where sufficient transport through the membrane must be balanced by a satisfactory immuno-protection of allogenic or xenogenic cells from the immune system of the recipient. In this work towards a biohybrid kidney it was tried to determine whether PAN and PSU polymers are able to allow a good proliferation and function of kidney epithelial cells. In this study we have also investigated the transport properties of the membranes investigated. It was shown that PSU is a typical nanofiltration membrane, while MC represents a typical microfiltration membrane. PAN has intermediate separation properties. Both PSU and PAN have immuno protective properties and would guarantee an immuno isolation of cells in a biohybrid organ from the host immune system.

Synthesis of polymers and formation of membranes can leave some toxic monomers or solvents in the bulk of
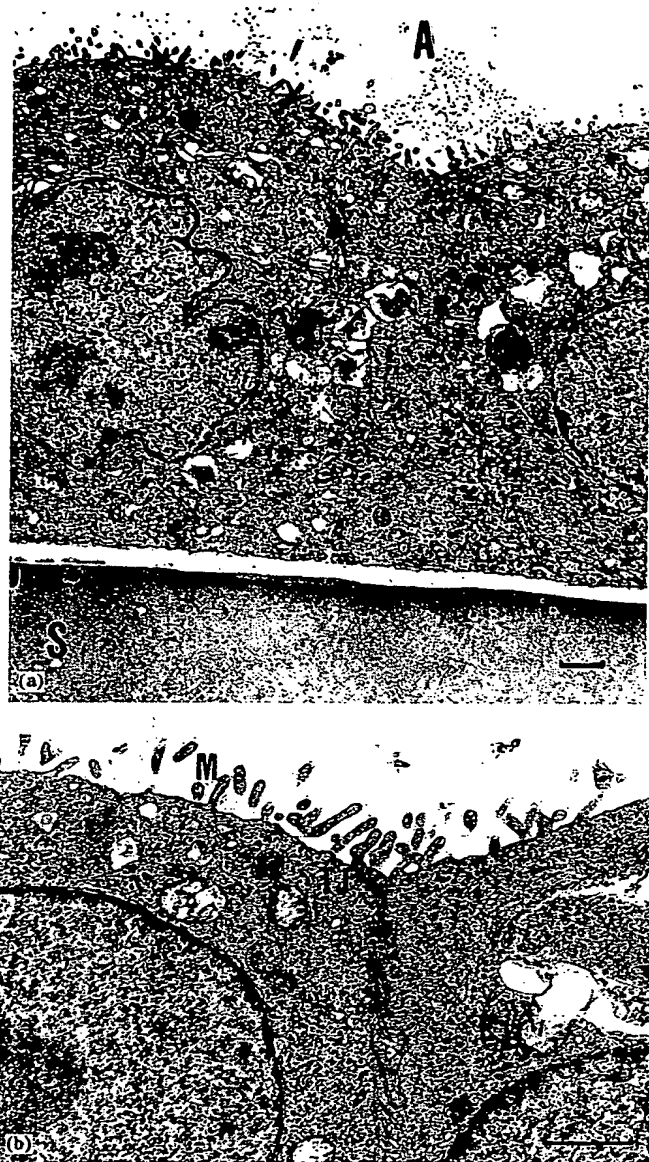
Fig. 8. Transmission electron micrograph of MDCK cells after 7 days of culture on the PSU membranes: (a) visualisation of loose attachment of epithelial cell sheet on the PSU support (S), artefacts (A). Bar 1.3 μm; (b) presence of tight junctions (TJ) and microvilli (M) showing an orientation of the cells with very tight cell–cell contacts over the whole contact area. Bar 0.9 μm.

the membrane [32]. Therefore, toxicity studies were carried out to measure the metabolic activity and membrane integrity of cells in contact with membrane extracts. It was shown that both membranes induced nontoxic action on fibroblasts. Studies on the attachment, morphology and proliferation of MDCK cells were carried out using mainly membranes and also spin coating films prepared from the different materials. Water contact angle measurements revealed that all membranes

provided moderate wettable surfaces with advancing contact angles of about 70°. A similar result was obtained when spin coating films prepared from the same polymers were investigated. The observed hysteresis between advancing and receding water contact angles indicates some heterogeneity of the surfaces caused by the affinity of the polymers for water molecules. From the results of our previous investigations using fibroblasts we have learnt that moderate wettable surfaces can provide suitable substrata for the attachment and proliferation of tissue cells [33]. Accordingly we observed, on PAN and PSU membranes, similar results as those on Millicell culture supports with the LDH assay. Since LDH can be used as a measure of the number of cells [30], we may conclude that PAN and PSU membranes provide similar conditions for the attachment and growth of kidney epithelial cells like commercial culture supports. Interestingly, insignificant differences between the smooth and rough side of the membranes were found with the LDH assay which was clearly observed measuring the metabolic activity of cells with the MTT assay. Here MDCK cells cultured on the smooth side express slightly decreased metabolic activity on PSU and PAN in comparison to MC. Dramatic decrease in the metabolic activity of cells, however, was observed when MDCK cells were cultured on the rough side of the membranes. From histology stainings (not shown here) it was detected that the highly porous structure of the rough side of the membranes prevented the formation of epithelial monolayers and decreased cell–cell contacts. This might have a negative impact on the functional state of cells with a decrease in the metabolic activity of cells.

Further studies on cellular viability and spreading were carried out with fluorescein diacetate staining of MDCK cells attached to spin coating films. The results were interpreted cautiously because of the differing water contact angles between films and membranes. However, it was shown that MDCK cells spread well on MC, PAN and PSU, which seems to be in accordance with the results of proliferation studies.

The transmission electron microscopy demonstrated that MDCK cells attached well to the smooth side of the PAN membrane, but much less intimate interaction was detected on the PSU membrane. Here, the epithelial layer and the substratum were well separated, indicating a rather weak interaction between cells and substratum. One explanation for this phenomenon is the presence of PVP in this membrane, which is known to inhibit cell adhesion [34]. However, from these ultra structural studies it also became obvious that the formation of close cell contacts and tight junctions was more intensive on PSU membranes, and not intensive on PAN. We conclude that a too tight binding of cells to the substratum, counteracts the cell–cell contacts which are crucial for the formation of epithelial sheets. On the other hand, measurements of transepithelial resistance—a measure of

epithelial barrier function [35]—has shown a similar specific functionality of MDCK cells as a solute barrier after 7 days of culture on all three different types of membranes. This has been reported recently by us [36].

In conclusion, this investigation has shown that polymers with superior membrane forming properties for the formation of hollow fibres such as PSU and PAN are suitable basic materials for the attachment and proliferation of MDCK cells which can be used for the development of a biohybrid kidney.

Acknowledgements

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